

ORAL PRESENTATIONS

Table 3 summarizes the expenses, number of registered case and estimated "real" cases, when 5% to 20% of the cases are registered.

Table 3. *The Danish society costs in 2002, human medicine and lost days of work, for pork related salmonellosis and yersiniosis assuming 5-20% of the real cases are registered.*

Infection	Reg. cases	Estimated no. of "real" cases	Estimated society expenses for "real" cases
Salmonellosis	77	385 – 1,540	165,300 – 601.100
Yersiniosis	192	960 – 3,840	324,300 – 1.392.000

Discussion: The veterinary *Salmonella* control program for swine have reduced the pork related number of human salmonellosis cases from 1,144 cases registered in 1993 to 77 cases in 2002. The annual expenses to the program were 6.9 million Euro in 2002. In total, the expenses to the veterinary program have been close to 90 million Euro since its initiation in 1995. Assuming that the number of human cases had remained at the pre-control level if the salmonella-program have not been implemented, the program have reduced the occurrence of human salmonellosis in Denmark by approximately 1,000 registered cases in 2002. The majority of human salmonellosis cases are not registered, as only the more severely affected patients are examined by culture, so the "real" number of salmonellosis cases avoided in 2002 was probably 5,000-20,000, equivalent to a saved society cost of at least 3.5-8.2 million Euro. Between 70–90% of the society cost to human salmonellosis and yersiniosis is due to lost working days. From a society point of view, the salmonella-program and the improved hygiene on slaughterhouses have significantly reduced the *Salmonella* and *Yersinia* related expenses to hospitalisation, consultation to physicians and lost days of work.

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Comparison of *Campylobacter coli* strains isolated from pigs and humans - porcine strains a possible source of human infection?

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Summary: The primary aim of this study was to detect and genotype *Campylobacter* strains from pigs and humans. AFLP (amplified fragment length polymorphism) analysis was used to compare

different genotypes to identify the genetic diversity of *Campylobacter coli* (*C. coli*) strains. Heterogeneous patterns were detectable among the porcine and human *C. coli* pool.

By using an optimized extraction method combined with a PCR it was possible to detect *C. coli* DNA in some samples of the investigated minced meat but it could not be distinguished between dead bacterial cells and viable but nonculturable cell (VBNC)-forms of *C. coli* strains.

Keywords: AFLP; minced meat; paramagnetic beads; PCR; VBNC

Introduction: *Campylobacter* spp. are one of the very common causes of infectious gastroenteritis in humans. In this respect *C. jejuni* is the most important species. But there is no sufficient information about the relevance and the source of human *C. coli* infection. *C. coli* is a commensal of the gut of pigs and is excreted in faeces.

1. In order to assess the importance of pork as a source of *C. coli*-induced campylobacteriosis in humans it was necessary to detect and evaluate the prevalences of *C. coli* in pigs and in humans.

2. To characterize the genetic relationship of human and porcine *C. coli* strains, the genotypes of these were compared.

3. The consumption of minced meat may be a possible source for human infection with *C. coli*. Therefore we investigated different minced meat products bacteriologically and also by two different molecular biological methods.

Materials and Methods: For isolation of *C. coli* in fattening pigs (n=1150) a rectal swab was placed directly into Bolton-Broth and then cultured on mCCDA. The enrichment broth and the plates were incubated microaerobically for 48 hrs at 42 °C.

After the bacteriological isolation of *C. coli* strains, the DNA was extracted using a DNA extraction kit and identified using the PCR-technique (Linton, Lawson, et al. 1997 125 /id)). Specific primers for the genus *Campylobacter* and the species *C. coli* were used.

For investigation of human *C. coli*, isolates were submitted to use from two regional laboratories as "thermophilic campylobacters" (n=456). Up to three single colonies from each agar plate were further processed by DNA extraction and PCR-technique described above.

In this study, AFLP was used for genetic typing of *C. coli* strains from pigs and humans as described previously (Duim et al., 1999).

To detect *C. coli* in pork products, minced meat was investigated bacteriologically and by PCR analysis. In addition an optimized PCR-technique was developed for a more sensitive detection of *C. coli* in minced meat. Boiled meat samples were mixed with a *C. coli* specific DNA-probe. After hybridisation paramagnetic beads were added to the mixture and incubated for 30 min at room temperature. *C. coli* specific PCR followed the extraction procedure.

Results: *C. coli* were detected in approximately 90 % of the fattening pigs in faeces (n=1150) and 21.5 % of human campylobacter cases bacteriologically examined (n=456). In the slaughter house we isolated *C. coli* at 85 % in faeces, 20 % on skin surfaces before chilling. After the chilling process no *C. coli* could be detected on the skin surfaces bacteriologically.

Human *C. coli* strains showed like porcine *C. coli* strains heterogeneous AFLP patterns. Nonetheless single similarities between human and porcine strains (isolated in the same region at similar times) could be established.

Culturable thermophilic *Campylobacter* spp. could not be detected in minced meat (n=125) bacteriologically. By direct PCR, the detection rate of *C. coli* increased slightly to 0.8 %.

When using an optimized extraction method combined with a PCR it was possible to detect *C. coli* DNA in higher numbers of the investigated minced meat samples.

Discussion: *C. coli* infection in humans is underestimated. At least 20 % of human campylobacteriosis are caused by *C. coli*. But the sources of *C. coli* infection in humans are not known.

The chilling process of pig carcasses in slaughter houses reduced the rate of culturable campylobacters on the carcass surface significantly.

Genotyping of *C. coli* revealed heterogeneous patterns among the human and porcine *C. coli* pool. It shows that different sources of infection in humans are most probable.

In minced meat we did not detect *C. coli* bacteriologically, but by the use of paramagnetic beads combined with PCR-technique we detected *C. coli* positive samples. This shows, that the meat was contaminated with *C. coli*. It is not clear what importance the presence of *C. coli* DNA in minced meat has for human infection, even though the presence of viable and culturable *C. coli* cells could be ruled out by bacteriological investigation. We could not distinguish between dead cells and VBNC forms of *C. coli* cells. The role of VBNC form of *C. coli*, a specific phenomenon of campylobacters (Lazaro et al., 1999) and certain other bacteria, has to be investigated further.

Conclusion: Porcine strains as sources of human *C. coli* infection can not be ruled out. Further research is needed to evaluate the *C. coli* findings in minced meat and the role of VBNC for human infection.

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Pathogenic bacteria and indicator organisms for anti-microbial resistance in pork meat at retail level in The Netherlands.

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Summary: Slaughter pigs and pork carcasses are often contaminated with pathogenic bacteria. Consequently raw meat on sale in retail stores may also contain these bacteria. In The Netherlands the calculated contribution by pigs to the relative occurrence of human salmonellosis in the period 1994-1998 was 25.2 % (van Pelt, 2001). Survey and monitoring data on the contamination of raw products with pathogens like *Salmonella*, *Campylobacter*, *Listeria monocytogenes* and *Escherichia coli* O157 are essential for making risk estimates, and the results of surveys carried out in 1990/2000 and 2002 are presented here. In 2002 also a surveillance of anti-microbial resistance among indicator bacteria (*Escherichia coli*, *Enterococcus faecium/faecalis*) isolated from pork meat was started. The results show that pork meat was contaminated with *Salmonella* in levels between 6.2 - 10.5 %, *S. Typhimurium* being the predominant serotype, and to a lesser extent with *Campylobacter*, *Listeria* and *E. coli* O157.

Keywords: *Salmonella*; *Campylobacter*; *Listeria monocytogenes*; *Escherichia coli* O157; *Enterococcus faecium/faecalis*.

Materials and methods: Raw meat products were sampled in the retail trade. Detection of *Salmonella*, *Campylobacter* and *Listeria monocytogenes* was carried out using standard methods. *E.coli* O157